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Epithelial modulation of cholinergic responses in rabbit trachea is partly due to neutral endopeptidase activity

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Abstract

By the simultaneous measurement of acetylcholine release and smooth muscle contraction in rabbit tracheal segments with and without epithelium, pre- as well as postsynaptic effects of this cell layer were studied on cholinergic neurotransmission. The epithelial cell layer exerted a presynaptic inhibitory influence on acetylcholine release, induced by KCl and electrical stimulation, with a concomitant decrease in the smooth muscle contractions. The responses elicited by exogenous acetylcholine, acting postsynaptically, were also inhibited in the presence of the epithelium. The epithelial effect was not accounted for by the production of inhibitory prostaglandins or a nitric oxide-synthase product. Furthermore, the epithelium did not function as a metabolic site for the degradation of acetylcholine. Phosphoramidon, an inhibitor of neutral endopeptidase, mimicked the effects of epithelium removal on the cholinergic responses to high frequency stimulation and on the acetylcholine-induced effects. Neutral endopeptidase inhibition did not further enhance the responses in epithelium-denuded segments. We therefore suggest that the inhibitory function of the epithelium can be partly explained by the activity of neutral endopeptidase, limiting the excitatory effects of tachykinins on cholinergic responses. An alteration in the neutral endopeptidase activity as a result of inflammatory responses and epithelial damage can contribute to the mechanism of airway hyperreactivity in asthma.

Keywords: Neutral endopeptidase; Acetylcholine; Epithelium; Trachea; Cholinergic neurotransmission; (Rabbit)

1. Introduction

Although the respiratory epithelium has long been considered to be an inert physical lining, it is nowadays believed to modulate airway smooth muscle tone in a wide variety of species. So far, three different aspects of epithelial function have been described (Morrison et al., 1990). The lining of the respiratory system acts as a barrier limiting access of agents to the smooth muscle layer, and protects the underlying sensory nerve endings from overstimulation by airborne stimulants. Furthermore, the epithelial cells have been shown to be a metabolic site for the uptake and/or degradation of a number of bronchoactive mediators (Farmer et al., 1986; Sekizawa et al., 1987; Lindström et al., 1991; Koga et al., 1992). Finally, bioassay experiments have demonstrated the release of an inhibitory factor by the epithelial cells. The identity of this factor is as yet unknown, although in some preparations an involvement of prostanoids has been suggested (Burgaud et al., 1993; Butler et al., 1987). Many controversies, however, still remain. Indeed, depending on the model, different stimuli were able to induce the inhibitory effect of the epithelial cell layer. Moreover, studies with canine and rabbit airways revealed prominent regional differences in the ability of the epithelium to influence smooth muscle tone along the bronchial tree (Hay et al., 1987; Raeburn et al., 1986). Therefore it is most likely that the epithelium exerts its modulatory effects through a number of mechanisms, which may vary according to the species studied, the airway diameter used and the pathological conditions imposed.

Epithelial damage is a common pathological finding in patients with asthma (Laitinen et al., 1985) and is brought in relation to airway hyperreactivity (Hogg and

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Eggleston, 1984; Allegra et al., 1989). The latter is considered as an exaggerated bronchoconstrictor response to a variety of stimuli, and because of the reversibility of this airway narrowing, smooth muscle contraction seems to play an important role in its development. As the parasympathetic nervous system is the most prominent bronchoconstrictor pathway (Barnes, 1986), we believe that more attention should be focused on the modulatory role of the epithelium on this neurotransmission.

Recently, we developed an in vitro model for the study of cholinergic neurotransmission in an isolated preparation of rabbit trachea, based on the simultaneous measurement of smooth muscle contraction and acetylcholine release after cholinergic stimulation (Loenders et al., 1992). We now examined whether the rabbit tracheal epithelium exerts a modulatory influence on these cholinergic responses, thereby making a distinction between possible pre- and postsynaptic effects.

2. Materials and methods

2.1. Experimental protocols

New Zealand white rabbits (2.5–3.5 kg) were anaesthetized with sodium pentobarbital (30 mg/kg i.v.) and exsanguinated via the carotid artery. The entire trachea was excised, cleaned of adhering adipose and connective tissue and cut into rings of approximately 70 mg weight. Segments of three different areas were combined to compensate for regional variability, and mounted in 2.5 ml organ chambers, filled with Krebs solution (composition in mM: NaCl 118.0; KCl 4.7; $CaCl_2$ 2.5; $MgSO_4$ 1.2; KH_2PO_4 1.2; $NaHCO_3$ 25.0; glucose 11.1), aerated with 95% O_2 and 5% CO_2 and maintained at constant temperature (37°C) and pH (7.4). The experiments were carried out in the presence of 1.5×10^{-6} M neostigmine to prevent breakdown of acetylcholine by acetylcholinesterase.

Epithelium removal was achieved by rubbing the lumen with a cotton-tipped applicator. By this method the epithelium was removed without apparent morphological damage to the underlying smooth muscle, as was confirmed by microscopic evaluation of sirius-red-stained paraffin-embedded tissue slices.

The segments were allowed to equilibrate for 2 h under an initial tension of 10 g. During this period the bathing fluid was exchanged 6 times at regular intervals.

Frequency-response curves (9 V, 2 ms, 5 min), as well as non-cumulative dose-response curves for acetylcholine, KCl and carbachol were constructed in the absence and presence of the epithelium. Changes in tension were measured isometrically.

When constructing the frequency-response curves and dose-response curves for KCl, the bathing fluid was collected on ice after each stimulation in order to estimate the evoked acetylcholine release with a sensitive and specific high pressure liquid chromatographic method with electrochemical detection.

The effects of possible inhibitors of the epithelial activity were also assessed. When present, they were added to the Krebs solution from the start of the equilibration period.

2.2. Estimation of acetylcholine

Acetylcholine in the bathing fluid was measured using a high pressure liquid chromatography method with electrochemical detection (Damsma et al., 1987; Deckers and Herman, 1988). This method is based on a high pressure liquid chromatographic separation of acetylcholine and choline on a reversed phase column, converted to a cation exchanger by lauryl sulphate (Chromspher C_{18} column, Chrompack). The mobile phase was a 0.2 M potassium phospate buffer (pH 8.0) at a flow rate of 0.6 ml/min. By reacting the column effluent with acetylcholinesterase and choline oxidase, immobilized on a postcolumn reactor (10×2.1 mm Imer, Chrompack), acetylcholine is consecutively hydrolized, and oxidized. The hydrogen thus produced is detected electrochemically (Pt electrode, 500 mV, BAS LC-4A amperometric detector, with an Ag/ AgCl reference electrode). Preparative sample workup was not necessary as none of the products used interfered with the assay. Calibration was carried out using pure acetylcholine as standard. The detector response was directly proportional to the amount of acetylcholine injected from 1 to 1000 pmol (Deckers et al., 1989). The detection limit of the assay was 10 pmol acetylcholine per gram of tissue. The intra-assay coefficient of variation was 2.0% ($n = 9$).

2.3. Drugs

The following drugs were used: acetylcholine hydrochloride (Sterop, Brussels, Belgium); neostigmine methylsulphate (Prostigmine, Roche, Brussels, Belgium); carbamylcholine; tetraisopropyl-pyrophosphoramide (iso-OMPA); phosphoramidon; capsaicine; N^{ω}-nitro-L-arginine methyl ester (L-NAME) (Sigma, St. Louis, MD, USA); indomethacine (Merck, Sharp and Dohme, München, Germany).

2.4. Statistics

Contractile responses are expressed as g contraction/g tissue. Release of acetylcholine is given as pmol

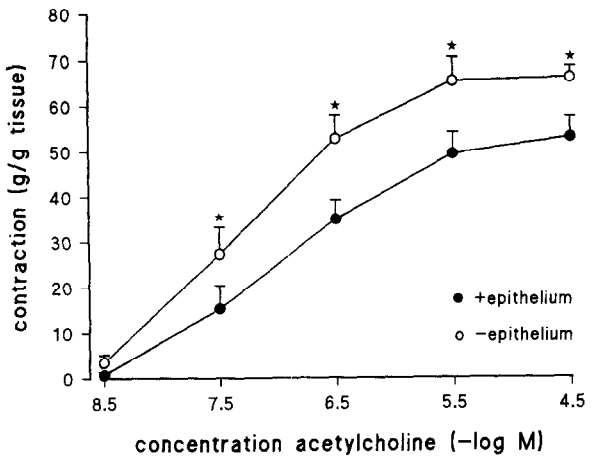

Fig. 1. Log concentration-response curves to acetylcholine in rabbit tracheal segments with (closed symbols) and without (open symbols) the epithelium. Results are the mean ± S.E.M. for six experiments. * $P < 0.05$.

acetylcholine/g tissue. The data are presented as mean values ± S.E.M. for the number of experiments indicated (number of experiments = number of animals).

The EF_{50} values were calculated by extrapolation as the frequency inducing 50% of the maximum contraction.

Student's paired *t*-test was used for statistical analysis. $P < 0.05$ was considered as significant.

3. Results

3.1. Influence of the epithelium

Epithelium removal potentiated the responses to acetylcholine, since the dose-response curve of the cholinergic neurotransmitter was shifted upwards after removal of the epithelium. The maximal contractile response to acetylcholine was significantly increased in the epithelium denuded segments (Fig. 1). However,

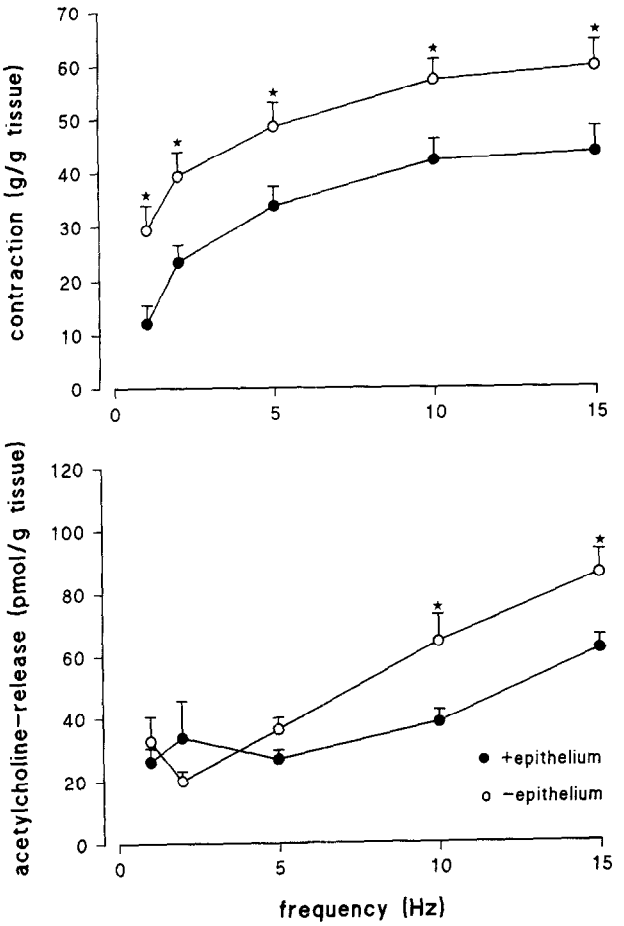

Fig. 2. Smooth muscle contraction (upper panel) and acetylcholine release (lower panel) to electrical field stimulation (9 V, 2 ms for 5 min) at different frequencies. Responses were measured in rabbit tracheal segments with (closed symbols) and without (open symbols) the epithelium. Results are the means ± S.E.M. for six experiments. * $P < 0.05$.

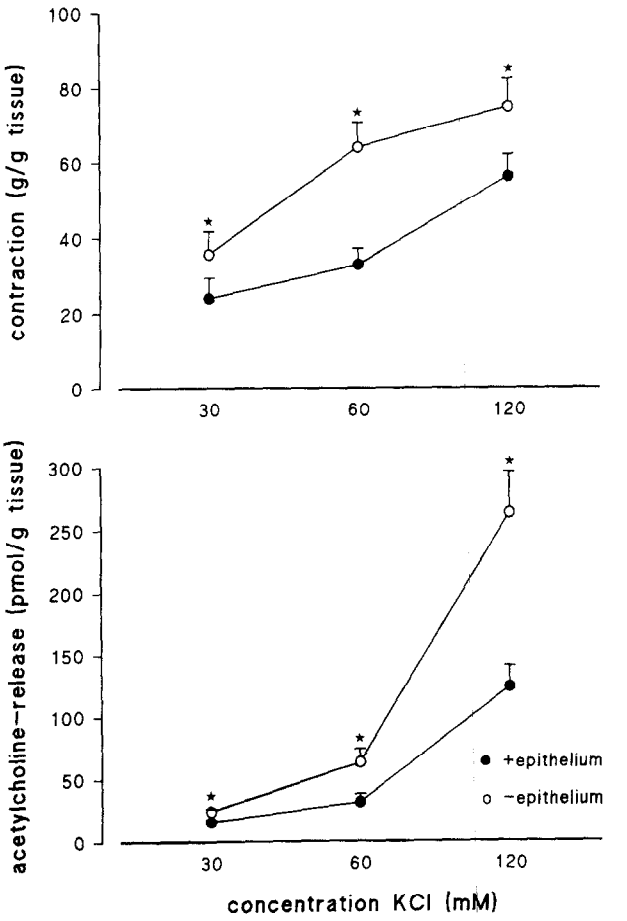

Fig. 3. Smooth muscle contraction (upper panel) and acetylcholine release (lower panel) to depolarising KCl solution in rabbit tracheal segments with (closed symbols) and without (open symbols) the epithelium. Results are the means ± S.E.M. for six experiments. * $P < 0.05$.

removing the epithelium did not alter the sensitivity to acetylcholine, as the EC_{50} values were unchanged (EC_{50} (+E) = 6.87 ± 0.24; EC_{50} (−E) = 7.11 ± 0.16).

As shown in Fig. 2, epithelium removal also enhanced the electrically induced contractions at all frequencies studied. The sensitivity to cholinergic stimulation (EF_{50} (+E) = 2.05 ± 0.21 Hz; EF_{50} (−E) = 1.05 ± 0.25 Hz, for $n = 6$), as well as the maximal contraction significantly increased.

At 10 and 15 Hz, the enhanced contraction was accompanied by a significant rise in the acetylcholine release, suggesting a presynaptic effect of the epithelium at these frequencies.

A depolarising KCl solution induced a dose-dependent acetylcholine output and smooth muscle contraction. The latter is partly due to the stimulation of muscarinic receptors by the acetylcholine released, since 75% of the contractile response to 60 mM KCl could be inhibited by 3×10^{-7}M atropine. Both KCl-induced effects were potentiated in the absence of the epithelial cell layer (Fig. 3).

3.2. Pharmacological characterisation

In order to characterise the nature of this epithelial inhibitory activity, experiments were carried out using various enzyme inhibitors.

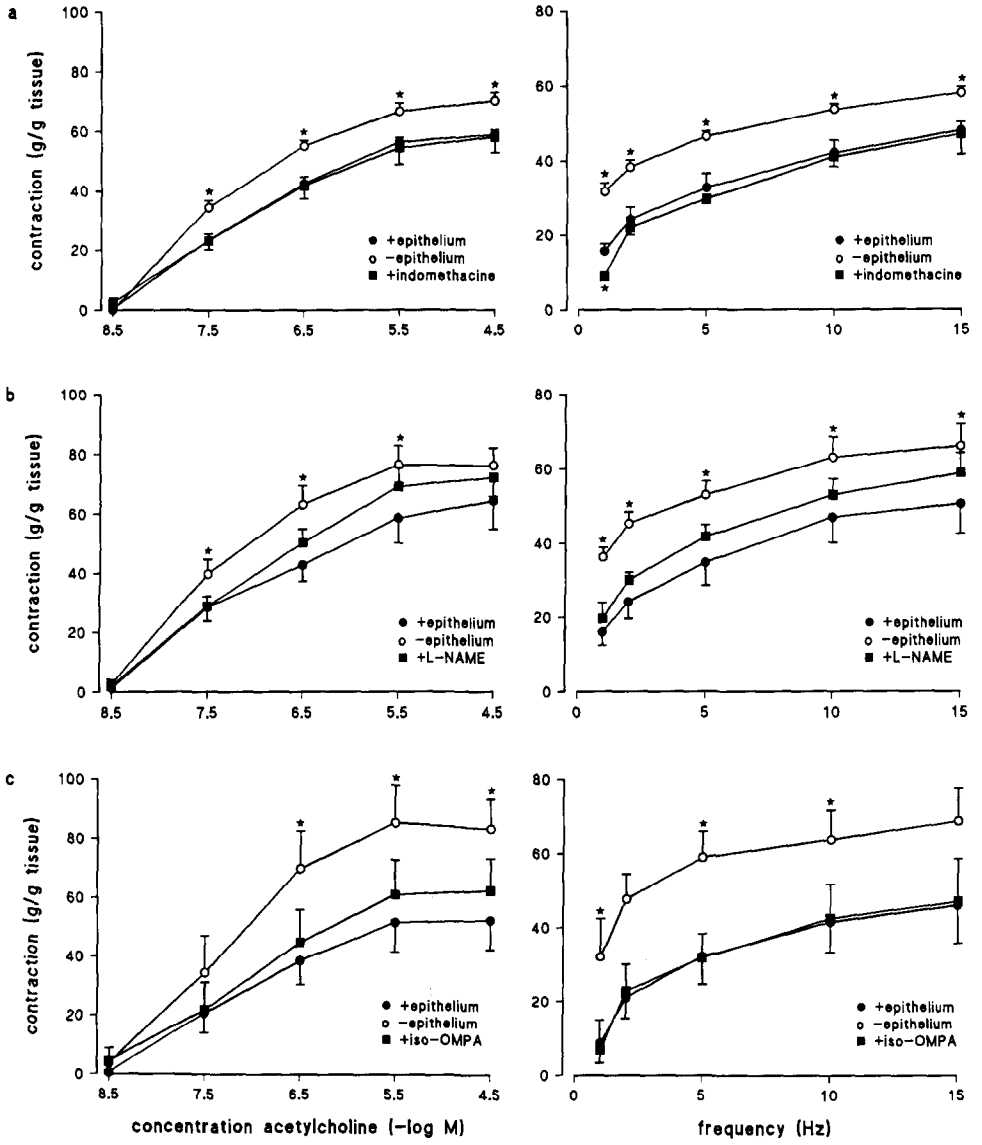

Fig. 4. Smooth muscle contraction to exogenous acetylcholine (left panel) and electrical stimulation (right panel) in rabbit tracheal segments with (closed symbols) and without (open symbols) the epithelium, in the absence (circles) and presence (squares) of 3×10^{-6} M indomethacine (a), 3×10^{-5} M L-NAME (b) and 10^{-4} M iso-OMPA (c). Results are the means ± S.E.M. for five or six experiments. * $P < 0.05$, versus intact segments.

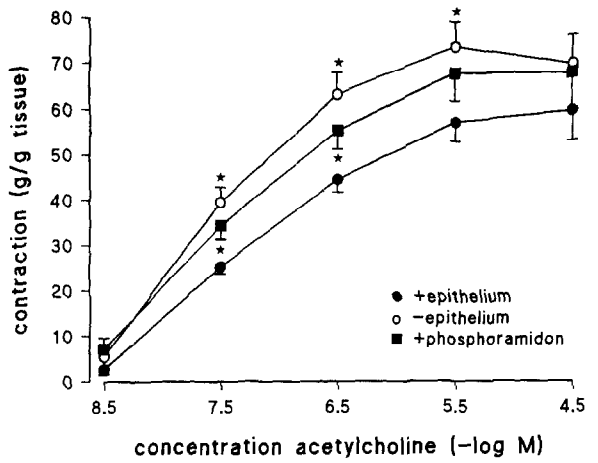

Fig. 5. Log concentration-response curves to acetylcholine in rabbit tracheal segments with (closed symbols) and without (open symbols) the epithelium, in the absence (circles) and presence (squares) of 10^{-5} M phosphoramidon. Results are the means ± S.E.M. for seven experiments. * $P < 0.05$, versus intact segments.

Inhibition of the cyclo-oxygenase with indomethacine (3×10^{-6} M) did not mimick the effect of epithelium removal on the dose-response curves to acetylcholine nor did it affect the frequency-response curves (Fig. 4a).

To study a possible involvement of nitric oxide (NO), N^{ω}-nitro-L-arginine methyl ester (L-NAME) was added to the bathing fluid in a concentration of 3×10^{-5} M. Blocking NO-synthase in intact preparations did not alter the cholinergic responses (Fig. 4b). An involvement of an NO-synthase product in our experimental conditions is therefore unlikely.

In order to test the hypothesis that the epithelium could be a metabolic site for acetylcholine, experiments were carried out using tetraisopropyl-pyrophosphoramide (iso-OMPA), which is known to block butyrylcholinesterase together with neostigmine which preferentially blocks acetylcholinesterase (Atack et al., 1989). This combination of cholinesterase inhibitors was un-

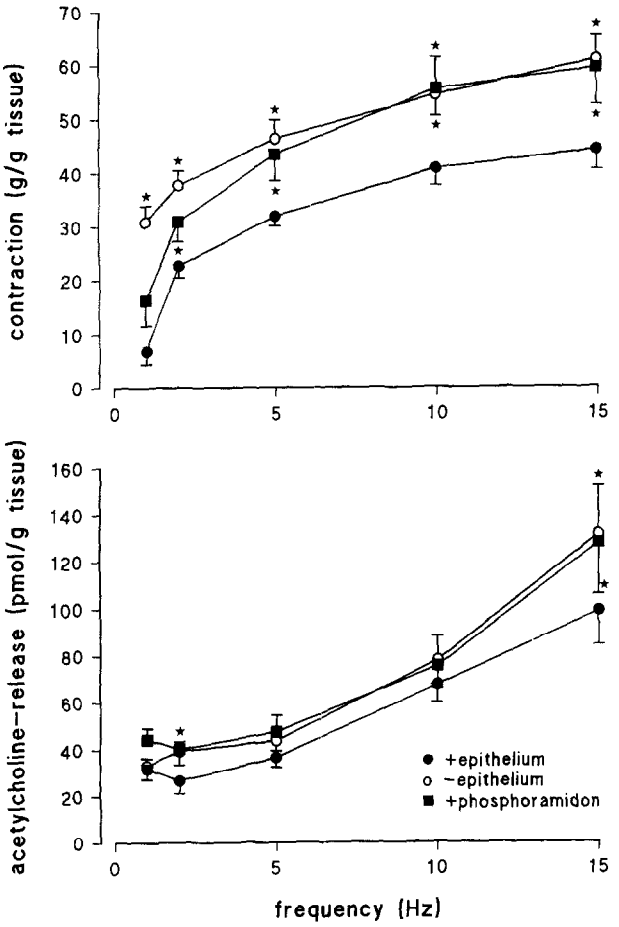

Fig. 6. Smooth muscle contraction (upper panel) and acetylcholine release (lower panel) to electrical field stimulation (9 V, 2 ms for 5 min) at different frequencies. Responses were measured in rabbit tracheal segments with (closed symbols) and without (open symbols) the epithelium, in the absence (circles) and presence (squares) of 10^{-5} M phosphoramidon. Results are the means ± S.E.M. for seven experiments. * $P < 0.05$, versus intact segments.

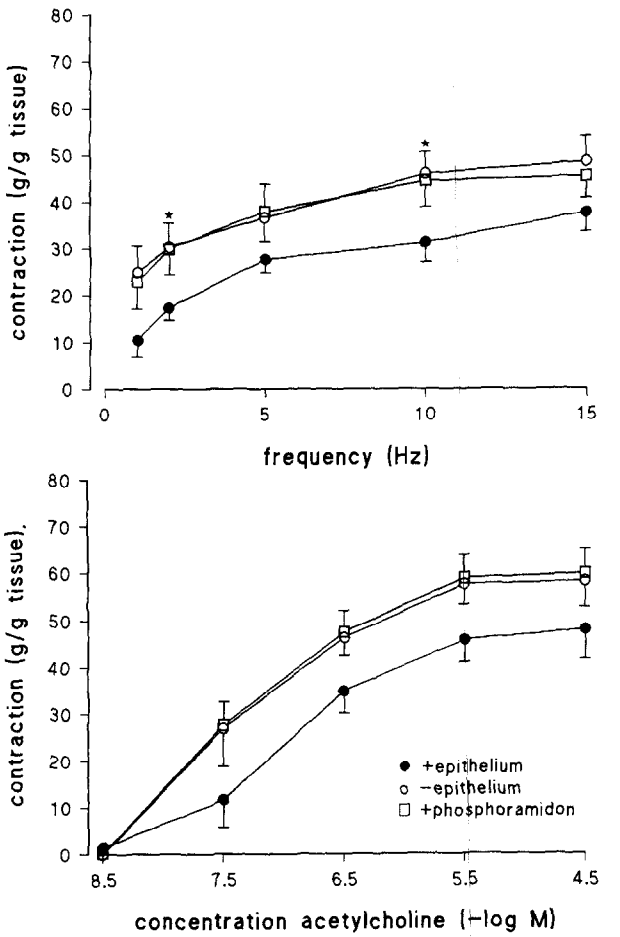

Fig. 7. Smooth muscle contraction to electrical stimulation (upper panel) and exogenous acetylcholine (lower panel) in rabbit tracheal segments with (closed symbols) and without (open symbols) the epithelium, in the absence (circles) and presence (squares) of 10^{-5} M phosphoramidon. Results are the means ± S.E.M. for four experiments. * $P < 0.05$, versus intact segments.

able to affect the electrically as well as acetylcholine-induced contractions (Fig. 4c). To further exclude an involvement of a cholinesterase activity of the epithelial cells, dose-response curves for carbachol, an acetylcholine analog that is not susceptible to enzymatic breakdown, were contructed. However, removal of the epithelium also significantly enhanced the sensitivity to carbachol (EC_{50} (+E) = 6.00 ± 0.19; EC_{50} (−E) = 6.62 ± 0.03 for $n = 6$, $P < 0.05$).

In intact segments, pretreatment with 10^{-5} M of the neutral endopeptidase inhibitor phosphoramidon (Dusser et al., 1989), significantly enhanced the responses to acetylcholine (3×10^{-7} M and 3×10^{-8} M) (Fig. 5). Moreover, at higher frequencies, phosphoramidon mimicked the effect of epithelium removal on the electrically induced contractions and acetylcholine release (Fig. 6). The observation that phosphoramidon did not further increase the electrically and acetylcholine-induced responses in segments without epithelium, suggests a predominant epithelial localisation of neutral endopeptidase (Fig. 7).

4. Discussion

Our results provide evidence that the rabbit tracheal epithelium exerts a modulatory effect on cholinergic activity. Acetylcholine release induced by both electrical stimulation at high frequencies and a depolarizing KCl solution, was inhibited by the epithelial cell layer with a concomitant reduction of the contractile responses. This is the first demonstration of a functional inhibitory effect of the rabbit tracheal epithelium on acetylcholine output. A presynaptic effect of the epithelial cells in guinea-pig trachea has already been suggested by Murlas (1986), and has been demonstrated by Wessler et al. (1990), albeit without measurements of smooth muscle contraction. The present results, however, are in contrast with the findings of Inoue et al. (1992) who did not observe an increased acetylcholine output after epithelium removal in rabbit tracheal strips. This discrepancy may relate to methodological differences since it is known that strips and rings from airways react differently to epithelial removal (Small et al., 1990).

Furthermore, the epithelium inhibited the contractile effects of endogenous acetylcholine, released at low frequency stimulation without altering the concomitant acetylcholine release. Also the responses to exogenous acetylcholine were enhanced in denuded segments as compared to intact segments.

These results are indicative for a postsynaptic inhibitory effect of the rabbit tracheal epithelium and are in agreement with the findings of others (Raeburn et al., 1986; Lev et al., 1990).

Therefore, in the rabbit trachea the epithelium modulates smooth muscle tone through at least two different mechanisms: one acting presynaptically and one acting at the postsynaptic level. Whether the pre- and postsynaptic effects elicited by KCl, electrical stimulation and exogenous acetylcholine, are mediated by one and the same mechanism remains to be determined.

Since epithelial damage is a common characteristic of asthmatic airways, this epithelium-dependent braking mechanism on the acetylcholine output and the acetylcholine-induced contractions may have clinical implications. It might, at least partly, explain the exaggerated bronchoconstrictor responses, which are a hallmark of the bronchial hyperreactivity. Moreover, there is reason to believe that in asthma there is a dysfunction of the muscarinic M_2-receptors which are involved in a presynaptic auto-inhibitory system, leading to an exaggerated acetylcholine output and subsequent smooth muscle contraction to cholinergic stimulation (Minette et al., 1989). Especially in these circumstances the epithelium may provide protection against this exaggerated bronchoconstriction. Kilbinger et al. (1991) indeed found an increase in contractile responses to cholinergic stimulation after presynaptic receptor blockade in epithelium-denuded segments in guinea-pig trachea.

Although a modulatory role for the lining of the respiratory tract on smooth muscle tone is now generally accepted, the mechanisms through which this occurs are still incompletely understood.

Some investigators were unable to demonstrate an epithelial effect on the activities of serosally applied mediators, and therefore relate the protective role solely to a passive barrier function of the epithelium (Holroyde, 1986). This is unlikely to be the case in our model since we observed inhibitory effects even though the smooth muscle surface of the preparations was exposed to the test agents. The finding that the responses to electrical stimulation were also smaller in intact preparations as compared to denuded segments, further excludes that the epithelium exerts its inhibitory effect by acting as a barrier for the spasmogens.

Bio-assay experiments revealed evidence for the release of an epithelium-derived relaxing factor (EpDRF) in response to bronchoactive mediators in several models (Vanhoutte, 1988; Bertrand and Tschirhart, 1993). The identity of this EpDRF is as yet unknown and might be stimulus- and species-dependent. In ferret (Ullman et al., 1990), guinea-pig (Hay et al., 1986; Burgaud et al., 1993) and canine trachea (Barnett et al., 1988), the involvement of a prostanoid mediating the epithelium-derived effects was suggested. Since the rabbit respiratory epithelium is a rich source for prostaglandin E_2 (Butler et al., 1987), a substance with

known effects on pre- and postsynaptic cholinergic activity (Deckers et al., 1989), we tested the influence of indomethacin in our model. However, no effect of this cyclo-oxygenase inhibitor was evident in the intact segments. This is in contrast to the results of Butler et al. (1987) who attributed the epithelial modulation of the betanechol-induced contractions to the release of an arachidonic acid metabolite. Because they used rabbit bronchi in their study, regional differences, which are reported to occur in rabbit airways (Raeburn et al., 1986) may explain these conflicting results.

In accordance to Güc et al. (1988) and Fernandez et al. (1989) in guinea-pig trachea and Spina and Page (1991) in rabbit bronchi, NO-synthase inhibition did not mimic the effects of epithelium removal in our experimental set up.

Another possible explanation for the observed epithelial effects is the presence of a metabolic site for acetylcholine different from the one which is sensitive to neostigmine. This attractive hypothesis was suggested by Koga et al. (1992) and could explain both the enhanced amount of acetylcholine recovered in the bathing fluid and the potentiation of the contractile responses. The presence of acetylcholinesterase in epithelial cells is rather controversial, and an involvement of this enzyme in our experiments is not to be expected anyway as they are conducted in the presence of the acetylcholinesterase inhibitor neostigmine. It might be possible however that butyrylcholinesterase is involved. But the butyrylcholinesterase inhibitor iso-OMPA was without any effect in intact segments, indicating that butyrylcholinesterase was not important for the degradation of the cholinergic neurotransmitter in rabbit trachea. Our observation that the epithelium also exerts an inhibitory effect on contractions to carbachol, a non-enzymatically degradable acetylcholine analog, further discards the possibility that the epithelium metabolizes acetylcholine.

Neutral endopeptidase is a membrane-bound enzyme which is present in the airway epithelium. It is important in the breakdown of many broncho- and vasoactive substances, including mediators such as tachykinins released from sensory nerves (Dusser et al., 1988). Inhibition of this enzyme by phosphoramidon increased the smooth muscle responses to high frequency stimulation. Also the acetylcholine release was potentiated by phosphoramidon. In epithelium-denuded segments, neutral endopeptidase inhibition did not further increase the electrically induced responses, confirming the predominant epithelial localisation of this enzyme (Solway and Leff, 1991). The acetylcholine-induced smooth muscle contraction was enhanced by neutral endopeptidase blockade as well. Our results suggest that neutral endopeptidase is implicated in the epithelium-dependent presynaptic inhibitory effects on the acetylcholine release. The postsynaptic inhibition at low frequencies of stimulation seems to be due to another, as yet unidentified, mechanism.

As neutral endopeptidase is reported not to degrade acetylcholine by itself (Di Maria et al., 1992), we suggest that this enzyme has an indirect influence on cholinergic neurotransmission. Evidence has been obtained for an excitatory effect of tachykinins on cholinergic activity, pre- as well as postsynaptically (Tanaka and Grunstein, 1984, 1986; Sakai et al., 1993). In this context, Inoue et al. (1992) have shown that epithelium removal increased the effects of exogenously applied substance P on acetylcholine release and electrically induced contractions, an effect that was apparently due to the removal of neutral endopeptidase. Therefore it is plausible that epithelium removal enhances the effects of excitatory tachykinins like substance P, resulting in an increased cholinergic activity. Although this might not be the sole mechanism through which the epithelium exerts its protective role, it might be an important one. The observation that allergen exposure or viral infections (Dusser et al., 1989) are associated with a diminished neutral endopeptidase activity, suggests an important contribution of the tachykinins in the development of bronchial hyperreactivity.

In conclusion, our results strengthen and enlarge the importance of neutral endopeptidase in limiting not only tachykinin-induced, but indirectly, also cholinergic responses and emphasize the role of the cholinergic neurotransmission in bronchial hyperreactivity.

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References

Allegra, L., L.M. Fabbri, G. Picotti and S. Mattoli, 1989, Bronchial epithelium and asthma, Eur. Respir. J. 2, Suppl. 6, 460s.

Atack, J.A., Q.-S. Yu, T.T. Soncrant, A. Brossi, S.J. Rapoport, 1989, Comparative inhibitory effects of various physostigmine analogs against acetyl- and butyrylcholinesterases, J. Pharmacol. Exp. Ther. 249, 194.

Barnes, P.J., 1986, Neural control of human airways in health and disease, Am. Rev. Respir. Dis. 134, 1289.

Barnett, K., D.B. Jacoby, J.A. Nadel and S.C. Lazarus, 1988, The effects of epithelial cell supernatant on contractions of isolated canine tracheal smooth muscle, Am. Rev. Respir. Dis. 138, 780.

Bertrand, C. and E. Tschirhart, 1993, Epithelial factors: modulation of the airway smooth muscle tone, Fundam. Clin. Pharmacol. 7, 261.

Burgaud, J.L., J. Javellaud and N. Oudart, 1993, Do perfused small caliber airways of guinea-pig release an epithelium-dependent relaxing factor?, Pulm. Pharmacol. 6, 217.

Butler, G.B., K.B. Adler, J.N. Evans, D.W. Morgan and J.L. Szarek, 1987, Modulation of rabbit airway smooth muscle responsiveness by respiratory epithelium, Am. Rev. Respir. Dis. 135, 1099.

Damsma, G., D. Lammerts van Bueren, B.H.C. Westerink and A.S. Horn, 1987, Determination of acetylcholine and choline in the femtomole range by means of HPLC, a post-column enzyme reactor and electrochemical detection, Chromatographia 24, 827.

Deckers, I.A. and A.G. Herman, 1988, HPLC detection of acetylcholine released from canine airway segments, Pflügers Arch. (Eur. J. Physiol.) 412, S42.

Deckers, I.A., M. Rampart, H. Bult and A.G. Herman, 1989, Evidence for the involvement of prostaglandins in modulation of acetylcholine release from canine bronchial tissue, Eur. J. Pharmacol. 167, 415.

Di Maria, G.V., M. Katayama, D.B. Borson and J.A. Nadel, 1992, Neutral endopeptidase modulates endothelin-1-induced airway smooth muscle contraction in guinea-pig trachea, Reg. Pept. 39, 137.

Dusser, D.J., E. Umeno, P.D. Graf, T. Djokic, D.B. Borson and J.A. Nadel, 1988, Airway neutral endopeptidase-like enzyme modulates tachykinin-induced bronchoconstriction in vivo, J. Appl. Physiol. 65, 2585.

Dusser, D.J., D.B. Jacoby, T.D. Djokic, I. Rubinstein, D.B. Borson and J.A. Nadel, 1989, Virus induces airway hyperresponsiveness to tachykinins: role of neutral endopeptidase, J. Appl. Physiol. 67, 1504.

Farmer, S.G., J.S. Fedan, D.W.P. Hay and D. Raeburn, 1986, The effects of epithelium removal on the sensitivity of guinea-pig isolated trachealis to bronchodilator drugs, Br. J. Pharmacol. 89, 407.

Fernandez, L.B., J.W. Paterson and R.G. Goldie, 1989, Co-axial bioassay of a smooth muscle relaxant factor released from guinea-pig tracheal epithelium, Br. J. Pharmacol. 96, 117.

Güc, M.O., M. Ilhan and S.O. Kayaalp, 1988, The rat anococcygeus muscle is a convenient bioassay organ for the airway epithelium-derived relaxant factor, Eur. J. Pharmacol. 148, 405.

Hay, D.W.P., S.G. Farmer, D. Raeburn, V.A. Robinson, W.W. Fleming and J.S. Fedan, 1986, Airway epithelium modulates the reactivity of guinea-pig respiratory smooth muscle, Eur. J. Pharmacol. 129, 11.

Hay, D.W.P., D. Raeburn and J.S. Fedan, 1987, Regional differences in reactivity and in the influence of the epithelium on canine intrapulmonary bronchial smooth muscle responsiveness, Eur. J. Pharmacol. 141, 363.

Hogg, J.C. and P.A. Eggleston, 1984, Is asthma an epithelial disease?, Am. Rev. Respir. Dis. 129, 207.

Holroyde, M.C., 1986, The influence of epithelium on the responsiveness of guinea-pig isolated trachea, Br. J. Pharmacol. 87, 501.

Inoue, K., Y. Sakai and I. Homma, 1992, An ubiquitous modulating function of rabbit tracheal epithelium: degradation of tachykinins, Br. J. Pharmacol. 105, 393.

Kilbinger, H., R. Schneider, H. Siefken, D. Wolf and G. D'Agostino, 1991, Characterization of prejunctional muscarinic autoreceptors in the guinea pig trachea, Br. J. Pharmacol. 103, 1757.

Koga, Y., S. Satoh, N. Sodeyama, Y. Hashimoto, T. Yanagisawa and Hirshman C.A., 1992, Role of acetylcholinesterase in airway epithelium inhibition of acetylcholine-induced contraction of guinea-pig isolated trachea, Eur. J. Pharmacol. 220, 141.

Laitinen, L.A., M. Heino, A. Laitinen, T. Kava and T. Haahtela, 1985, Damage of the airway epithelium and bronchial reactivity in patients with asthma, Am. Rev. Respir. Dis. 131, 599.

Lev, A., G.C. Christensen, R. Zhang and S.G. Kelsen, 1990, Epithelial effects on tracheal smooth muscle tone: influence of muscarinic antagonists, Am. J. Physiol. 258, L52.

Lindström, E.G., R.G.G. Andersson, G. Granérus, N. Grundström, 1991, Histamine receptors, distribution and metabolism: Is the airway epithelium responsible for histamine metabolism in the trachea of guinea-pigs?, Agents Actions 33, 170.

Loenders, B., M. Rampart and A.G. Herman, 1992, Selective M_3 muscarinic receptor antagonists inhibit smooth muscle contraction in rabbit trachea without increasing the release of acetylcholine, J. Pharmacol. Exp. Ther. 263, 773.

Minette, P.A., J.W. Lammers, C.M. Dixon, M.T. McCusker and P.J. Barnes, 1989, A muscarinic agonist inhibits reflex bronchoconstriction in normal but not in asthmatic subjects, J. Appl. Physiol. 67, 2461.

Morrison, K.J., Y. Gao and P.M. Vanhoutte, 1990, Epithelial modulation of airway smooth muscle, Am. J. Physiol. 258, L254.

Murlas, C., 1986, Effects of mucosal removal on guinea-pig airway smooth muscle responsiveness, Clin. Sci. 70, 571.

Raeburn, D., D.W.P. Hay, V.A. Robinson, S.G. Farmer, W.W. Fleming and J.S. Fedan, 1986, The effect of verapamil is reduced in isolated airway smooth muscle preparations lacking the epithelium, Life Sci. 38, 809.

Sakai, Y., K. Inoue and I. Homma, 1993, Relations of contractile hyperreactivity to tachykinins in rabbit trachea, Reg. Pept. 46, 233.

Sekizawa, K., J. Tamaoki, J.A. Nadel and D.B. Borson, 1987, Enkephalinase inhibitor potentiates substance P-induced and electrically induced contraction in ferret trachea, J. Appl. Physiol. 63, 1401.

Small, R.C., D.M. Good, J.S. Dixon and I. Kennedy, 1990, The effects of epithelium removal on the actions of cholinomimetic drugs in opened segments and perfused tubular preparations of guinea-pig trachea, Br. J. Pharmacol. 100, 516.

Solway, J. and A.G. Leff, 1991, Sensory neuropeptides and airway function, J. Appl. Physiol. 71, 2077.

Spina, D. and C.P. Page, 1991, The release of a non-prostanoid inhibitory factor from rabbit bronchus detected by co-axial bioassay, Br. J. Pharmacol. 102, 896.

Tanaka, D.T. and M.M. Grunstein, 1984, Mechanisms of substance P-induced contraction of rabbit airway smooth muscle, J. Appl. Physiol. 57, 1551.

Tanaka, D.T. and M.M. Grunstein, 1986, Effect of substance P on neurally mediated contraction of rabbit airway smooth muscle, J. Appl. Physiol. 60, 458.

Ullman, A., C.-G. Löfdahl, N. Svedmyr and B.-E. Skoogh, 1990, Nerve stimulation releases mucosa-derived inhibitory factors, both prostanoids and nonprostanoids, in isolated ferret trachea, Am. Rev. Respir. Dis. 141, 748.

Vanhoutte, P.M., 1988, Epithelium-derived relaxing factor(s) and bronchial reactivity, Am. Rev. Respir. Dis. 138, S24.

Wessler, I., D. Hellwig and K. Racké, 1990, Epithelium-derived inhibition of [^{3}H] acetylcholine release from the isolated guinea-pig trachea, Naunyn-Schmied. Arch. Pharmacol. 342, 387.